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STUDIES OF THE ECDYSIOTROPIC ACTIVITY OF JUVENILE HORMONE IN PUPAE OF THE TOBACCO HORNWORM, *MANDUCA SEXTA*

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Abstract

Topical administration of a juvenile hormone analog (JHA) to pupae of the tobacco hornworm accelerated the initiation of adult development and proved to be an ecdysiotropic stimulus even in the absence of the brain. Nevertheless, individuals developing in response to JHA were never normal. In fact, doses of JHA too low to accelerate development often provoked typical abnormalities in the resulting moths. Allatectomy of either diapause-destined or non-diapause pupae failed to alter the time course of adult development. In line with this finding, corpora allata (CA) from both diapause-destined and non-diapause pupae were without activity in two juvenile hormone (JH) bioassays. These results suggest that JH is not involved in the normal initiation of adult development. Indeed, it must be absent for normal development to proceed. Evidently, the ecdysiotropic effect of exogenous JH in pupae reflects a sensitivity to JH retained from the larval period.

Introduction

Increased secretion of ecdysone by the prothoracic glands (PG) is believed to constitute the proximate stimulus for the termination of pupal diapause and initiation of adult development. This augmented synthesis is believed to depend on the production of an ecdysiotropic hormone by the pupal brain, the so-called prothoracicotropic hormone (PTTH). Thus, in many well-documented instances, removal of the brain can significantly delay or even prevent the onset of adult development (for review, see Safranek and Williams, 1980).

In previous work from this laboratory we have examined the regulation of adult development in pupae of the tobacco hornworm, *Manduca sexta*. In these studies we have documented the critical role of the brain and PG in the normal initiation of adult development in both diapausing and non-diapausing pupae (Safranek and Williams, 1980). But in other studies our findings conflict with the classical model. For example, we have observed the initiation of adult development in brainless or decapitated pupae as well as in pupae lacking PG (Safranek and Williams, 1980; Safranek *et al.*, 1986). We have also demonstrated the ability of juvenile hormone (JH) or its analogs (JHA) to hasten the termination of pupal diapause and the initiation of adult development (Safranek *et al.*, 1980). These findings suggested that the endocrine system governing adult development in the hornworm might be significantly different from that defined by the classical model. In the present study we focus especially on

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Abbreviations: CA = corpora allata; JH = juvenile hormone; JHA = juvenile hormone analog; LD = long-day; SD = short-day; PG = prothoracic gland; PTTH = prothoracicotropic hormone; RIA = radioimmunoassay.

the ability of exogenous JH to accelerate the termination of pupal diapause and examine its possible role in the normal onset of adult development.

Materials and Methods

Hornworms were reared as described previously (Safranek and Williams, 1980) under either a short-day (SD, 12L:12D) photoperiod or a long-day (LD, 17L:7D) photoperiod at 25°C. The day of pupation is termed Day 1 of the pupal stage, and the first seven days of the pupal stage Week 1. Operations and ligations were performed as described previously (Safranek and Williams, 1980). Allatectomy was performed through a small horizontal incision in the vertex of the head within 12 h after pupal ecdysis. The corpora cardiaca were left *in situ* in this procedure. Sham operations on the CA were performed with complete visualization of these glands. In all experiments the initiation of development was recognized under the dissecting microscope by tracheal apolysis in the pupal wings.

The JHA "Hydroprene" (ZR-512, Zoecon Corp., technical grade) was used in certain experiments. For topical application, it was dissolved in acetone and dispensed onto the thorax with a 100 μl Hamilton syringe on a repeating dispenser.

The endocrine activity of individual CA was evaluated by the use of the "black larval assay" as described by Safranek and Riddiford (1975). JH secretion was also bioassayed by the implantation of CA into the heads of non-diapausing LD pupae within 24 h after pupal ecdysis: After the completion of development, the moths were scored for JH-induced morphological aberrations of the type described by Riddiford and Ajami (1973).

Ecdysteroid levels were measured by radioimmunoassay as previously described (Carrow *et al.*, 1981) using 20-OH-ecdysone as the standard.

Results

Effects of juvenile hormone application on diapausing pupae

Groups of 50 diapause-destined SD pupae received one of three graded doses of JHA in a single topical application on the first day after pupal ecdysis. A control group of 50 similar pupae was treated with the acetone solvent only. The initiation of adult development was monitored at weekly intervals.

As shown in Figure 1, the two lower doses of JHA failed either to accelerate or to retard development over and beyond that of the controls. By contrast, about half of the individuals that received the highest dose of 200 μg terminated diapause prematurely; the rest, which were not distinguished by their sex, did not develop notably faster than the controls. Additional experiments demonstrated that *all* individuals initiated development within 3 weeks of pupation after a single administration of 400 μg (n = 20) or after daily applications of 20 μg over 7 days (n = 15).

Those pupae initiating development during the first 6 weeks in response to the high dose of 200 μg uniformly displayed pronounced JH-related abnormalities of the type previously described by Riddiford and Ajami (1973). These abnormalities were most reliably apparent in the compound eyes where the occurrence of facet-free crescents regularly provided clear evidence of an individual's exposure to JHA. Even when the dose was too low to accelerate the termination of diapause, as in the case of pupae receiving a single dose of 20 μg, those individuals which initiated development within the first 4 weeks after pupation showed typical JH-induced abnormalities, often limited to the compound eyes.

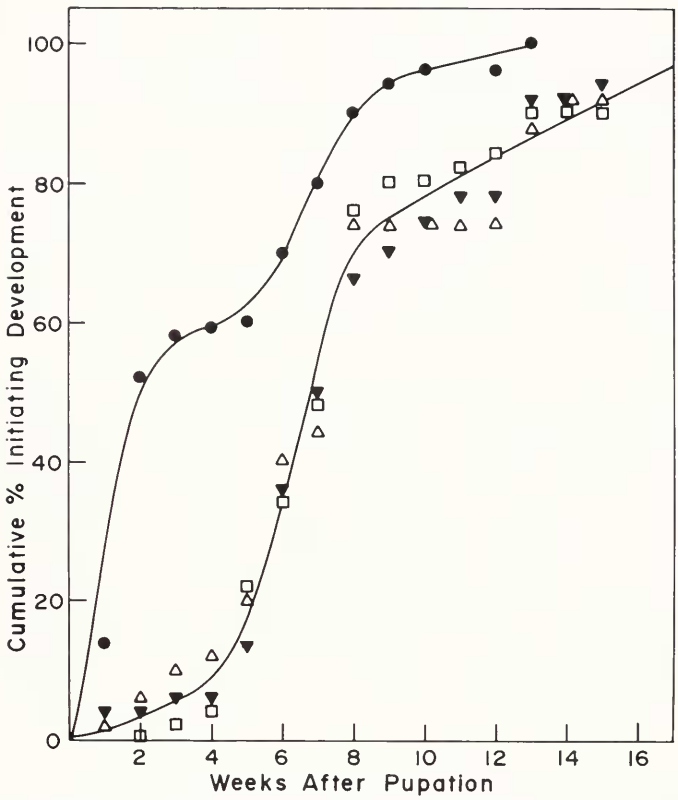

FIGURE 1. Initiation of development by groups of short day, diapause-destined pupae receiving topical application of JHA on the day of pupal ecdysis. Dosages of hormone per pupa were 200 μg (●), 20 μg (▼), 2 μg (□). Hormone was delivered in 2 μl acetone and one group of pupae received topical application of 2 μl acetone only (△). Pupae were examined for tracheal apolysis at weekly intervals. The cumulative percentage of each group demonstrating apolysis is plotted as a function of time after pupation. The lines are drawn by inspection.

Ecdysiotropic effects of JHA

We next inquired whether the acceleration of development witnessed in preparations treated with JHA was accompanied by an elevation of the ecdysteroid titer. Three groups were established: intact diapause-destined pupae, sham-operated diapause-destined pupae, and diapause-destined pupae from which the brain had been extirpated on Day 2. To individuals in each of these groups we administered daily topical applications of 20 μg of JHA beginning on pupal Day 3 and continuing until the onset of adult development. Hemolymph was collected on the day that tracheal apolysis was first observed and assayed for ecdysteroids by RIA. Ecdysteroid levels were also measured in hemolymph from two JHA-free control groups, one consisting of 4-week-old diapausing pupae, the other of individuals that had been in diapause for at least 4 weeks and had undergone tracheal apolysis during the 24 h preceding hemolymph collection.

Intact JHA-treated pupae initiated development 5–14 days after initiation of

TABLE I

Effects of JHA administration on the ecdysteroid titer at the outset of adult development

Procedure	Stage	Number	Ecdysteroid titer*
SD pupa, intact	Pupal diapause, 4 weeks	12	0.23 ± 0.05
SD pupa, intact	Tracheal apolysis	12	19 ± 7
SD pupa, intact + JHA	Tracheal apolysis	12	43 ± 12
SD pupa, sham + JHA	Tracheal apolysis	5	49 ± 15
SD pupa, − brain + JHA	Tracheal apolysis	10	30 ± 15

* The ecdysteroid titer is expressed in μg/ml 20-OH-ecdysone equivalents.

treatment, 50% by the seventh day. Sham-operated individuals developed 7–18 days after initiation of treatment, 50% by the ninth day. All brainless individuals developed 5–35 days after initiation of treatment, 50% by the eleventh day. The ecdysteroid titers of these groups and of the control pupae are shown in Table I. Manifestly the JHA treatment had a marked ecdysiotropic effect on all individuals. The average ecdysteroid titer achieved at the outset of development was two orders of magnitude higher than that typical of diapausing pupae. Moreover, the ecdysteroid levels noted in these groups were higher even than those typically attained by intact untreated pupae at a similar developmental stage. This was true even of brainless JHA-treated pupae. No correlation was seen between the ecdysteroid titer and the day of treatment on which development was first noted.

Effects of allatectomy

The CA were removed from 50 diapause-destined SD pupae 6–12 h after pupal ecdysis. Another 50 were sham-operated, while an additional 100 served as unoperated controls. As shown in Figure 2, all three groups underwent a typical and essentially identical diapause. Once apolysis was initiated, the individuals of all three groups developed into morphologically normal adults in the usual period of 3 weeks. A duplicate experiment on three groups of 25 pupae provided substantially the same results.

The experiment was repeated on three groups of potentially non-diapausing LD pupae. The results are summarized in Figure 3. Unoperated controls initiated development in an average of 4.1 days, sham-operated controls in 5.5 days, and allatectomized pupae in 6.6 days. The one day delay of allatectomized pupae relative to controls could have been due to the slightly greater injury involved in the allatectomy. It could also reflect the putative role of the CA as neurohemal organs in this species (Nijhout, 1975; Gibbs and Riddiford, 1977; Agui *et al.*, 1980; Carrow *et al.*, 1981).

Assay of the JH activity of pupal corpora allata

Secretion of JH by pupal CA was assayed in *bl* larvae and in non-diapausing pupae as described in Materials and Methods. To this end, groups of 12 non-diapausing pupae received CA from Day 1 or Day 3 non-diapausing pupae or from Day 1 or Day 14 diapause-destined pupae. Each assay pupa received one pair of CA. All individuals subsequently developed into morphologically normal adults—a result documenting the inactivity of the pupal CA. By contrast, implantation of only a

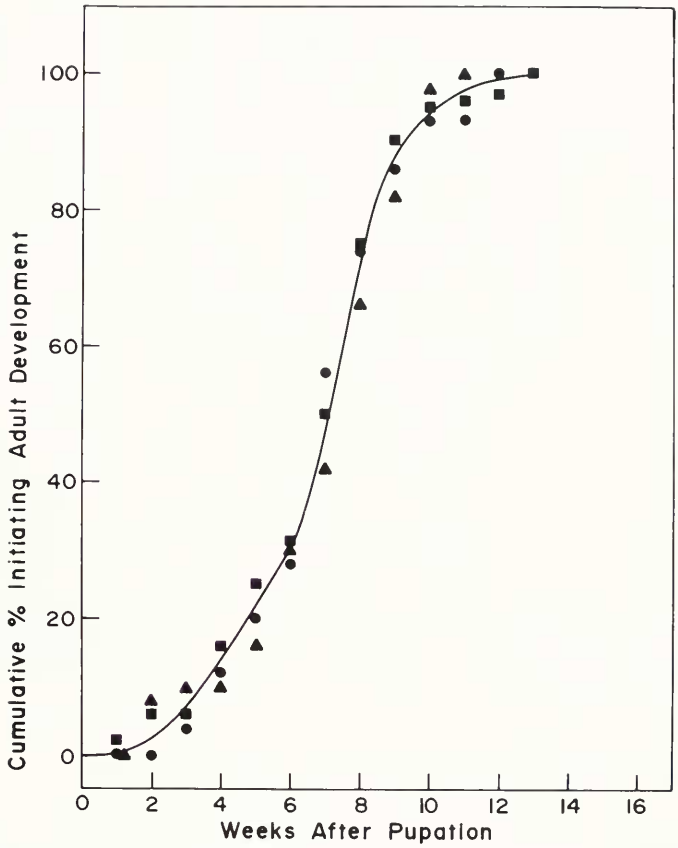

FIGURE 2. Lack of effect of allatectomy on the termination of pupal diapause. Diapause-destined (SD) pupae were operated 6–12 h after pupal ecdysis and were examined for initiation of development at weekly intervals. The three groups consisted of 50 allatectomized pupae (■), 50 sham-operated pupae (●), and 100 unoperated controls (▲). The cumulative percentage of each group showing apolysis is plotted as a function of time after pupation. The line is drawn by inspection.

single CA from a Day 1 fifth-instar LD larva into each of 12 additional non-diapausing pupae produced a range of typical JH-related aberrations in all resulting adults.

In additional tests, individual CA from the same classes of donors as above were implanted for bioassay into appropriate *b1* larvae. The average score of each group of 12 pupal CA was less than 0.5, indicating essentially no detectable activity. By contrast, the CA from a group of 12 early fifth-instar larvae averaged an essentially maximal score of 2.8. Thus, whereas CA from larvae at the outset of the fifth instar were highly active in both assays, none of the pupal CA possessed any detectable JH activity when similarly tested.

DISCUSSION

The foregoing findings leave little doubt that elevation of the ecdysteroid titer is the ultimate cause of the onset of adult development in pupae subjected to juvenoid

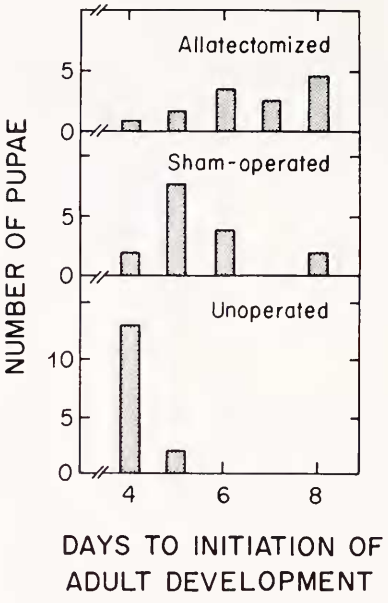

FIGURE 3. Effect of allatectomy on the initiation of development by non-diapausing (LD) pupae. Operations were performed 6–12 h after pupal ecdysis. Individuals were examined daily for tracheal apolysis. Each of the three groups comprised 15–18 pupae.

exposure, just as in the case of untreated pupae. Thus, as illustrated in Table I, the ecdysteroid titer in JHA-treated preparations at the outset of adult development achieved average levels 100 times that typical of untreated diapausing pupae, and in fact exceeded that encountered in untreated individuals spontaneously initiating adult development. Since this same result was observed in brainless pupae, the brain was manifestly unnecessary for mobilizing ecdysone sufficient to provoke the developmental response. Moreover, since the ecdysteroid titer at the outset of JHA-provoked development was actually greater than that witnessed normally, the acceleration of development occurring after JHA administration cannot be attributed to an enhanced tissue sensitivity to ecdysteroids, as has been suggested (Sehnal *et al.*, 1981).

The most parsimonious hypothesis is that the administered juvenoid acted directly on the PG to provoke the secretion of ecdysone. Nevertheless, this possibility has been questioned in the literature. Thus, the suggestion has been made that JH acts indirectly by mobilizing a diffusable factor from the fat body which in turn enhances ecdysone secretion by the PG; a direct action on the PG has been specifically denied (Gruetzmacher *et al.*, 1984). Other experiments have been interpreted to indicate an effect of JH on PG through an indirect mechanism requiring the PG to be *in situ* (Sehnal *et al.*, 1981). The question of direct or indirect action of JH on the PG remains, in our opinion, unresolved; we shall consider it in further detail in a subsequent communication.

Though JH can accelerate the initiation of adult development of diapausing *Manduca* pupae and has been shown to have pronounced ecdysiotropic effects at this stage, the hormone appears to play no role in normal adult development or in the maintenance or termination of diapause. Indeed, JH must be absent for normal de-

velopment to proceed. Thus as we have seen, the initiation of adult development could not be stimulated by JHA without also producing morphological aberrations in the resultant adults. Indeed, abnormalities could be brought about by doses of JH that were too low to accelerate development. In addition, as summarized in Figures 2 and 3, allatectomy produced virtually no delay in the development of either diapausing or non-diapausing pupae. Finally, pupal CA proved to be completely inactive in two different bioassays for JH. In conjunction with our previous results (Safranek and Williams, 1980) these findings make clear that the pupal brain's ecdysiotropic effects are not mediated through activation of JH production by the pupal CA.

The present findings confirm and extend previous observations of Bradfield and Denlinger (1980) that allatectomy failed to alter the duration of the *Manduca* pupal diapause. Moreover, in that same study JH could not be detected in the hemolymph derived from diapausing pupae of several different ages—a finding consistent with the inactivity of pupal CA documented in the present investigation. The current results also agree with prior findings documenting the lack of a role for JH in the initiation of adult development in silkworm pupae (Ichikawa and Nishiitsutsuji-Uwo, 1959; Williams, 1959, 1961; Gilbert and Schneiderman, 1959).

One remaining question concerns the *raison d'etre* for the marked sensitivity to juvenoids during the pupal stage. Though the application of JHA can accelerate the termination of pupal diapause, the aberrant development induced by even low levels of JH makes clear that at least the early phases of adult morphogenesis require a JH-free environment. The pupal epidermis has long been known to retain from the larval stage the ability to respond to exogenous JH at the outset of adult development. Similarly the ecdysiotropic effects of JH administered during the pupal stage may reflect a normally unused sensitivity carried over from the larval period when JH is typically present and may have significant effects on ecdysone secretion.

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